This pressure is reduced by 30% when standing, and by 50% in the reclining position. In cadavers and anesthetized patients with fully relaxed muscles, resting pressures are low compared with resting but awake subjects, for when a subject is awake the spinal muscle tone produces compressive force on the discs[10], whereas in sleep muscle tone decreases, especially during REM sleep. These factors are borne out by study I, with continuing loss in stature during deprivation of sleep. Not until subjects slept, and their muscle tone diminished, was accumulated loss regained. Precision of measurement is less in the subdivisions of the vertebral column, causing difficulty in establishing any pattern of change in cervical, thoracic and lumbar regions. Discrepancy between overall change in stature and sum of the changes in back lengths could indicate compression in the leg, through apposition of the bones of the leg and foot[15]. A small gain in stature has been shown following a rest without sleep[7], when pressure on cartilage would be reduced, but it would appear that full return of stature lost by day is dependent on sleep.

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2 Buffon, G. L. L., Histoire Naturelle Générale et Particulière, vol. 17, p. 301 (1777). Ed. C. S. Sonnini. Paris 1800.
3 Bradford, E. H., Boston Med. Surg. J. *109* (1883) 246.
4 Curtiss, F. H., Am. phys. Educ. Rev. *3* (1898) 270.
5 Weiner, C., Verh. naturw. Ver. Karlsruhe *11* (1888–1895) 98.
6 Backman, G., C. r. Soc. Biol. *90* (1924) 1118.
7 Goode, J. D., and Theodore, B. M., Engineering Med. *12* (1983) 99.
8 Palmer, C. E., Anat. Rec. *45* (1930) 234.
9 De Pukey, P., Acta orthop. scand. *6* (1935) 338.
10 Duthie, R. B., and Ferguson, A. B., Mercer's Orthopaedic Surgery, 7th edn, p. 880. Arnold, London 1973.
11 Davenport, C. B., Steggerda, M., and Drager, W., Proc. Am. Acad. Arts Sci. *69* (1934) 265.
12 Weiner, J. S., and Lourie, J. A., Human Biology. A guide to field methods, p. 8. Blackwell Scientific Publ. 1969.
13 Nachemson, A., and Morris, J. M., J. Bone Jt Surg. *46A* (1964) 1077.
14 Palmer, C. E., Sch. Phys. Bull. *3* (1933) 3.

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Freezing-tolerance in the woodroach *Cryptocercus punctulatus* (Scudder)

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Summary. Winter-acclimated *Cryptocercus punctulatus* are able to withstand ice crystal formation within their bodies (freeze-tolerant), and contain hemolymph plasma ice nucleating factors (INF's) throughout the year. In addition, ribitol, a sugar alcohol accumulates in their hemolymph during winter. This represents a new report of INF's occurring in the Dictyoptera, and the presence of ribitol in the hemolymph of the Insecta.
Key words. Freeze-tolerance; ribitol; *Cryptocercus punctulatus* (Scudder).

Wood cockroaches *(Cryptocercus punctulatus)* are primitive insects which inhabit downed, decaying logs in scattered mountainous regions of North America. Much like termites, they consume wood and utilize cellulose as a food source with the aid of symbiotic gut protozoans[1,2]. Mated pairs remain together in the same log for 4–5 years, sharing in the care (brooding) of their young which mature in 6–7 years and may live for 10 years[3]. Since these insects are found at relatively high elevations, studies were conducted to evaluate the ability of these insects to survive cold temperatures.

Insect overwintering biology has been of interest for many years. Information on insect cold-tolerance and cryobiology has increased since Chino[4] reported the occurrence of glycerol and sorbitol in cold-acclimated *Bombyx mori* eggs. Most early studies focused on the production and effects of glycerol and other polyols in insect hemolymph. These materials usually function as antifreeze agents by lowering the hemolymph supercooling point providing insects with protection from freezing.

Zachariassen and Hammel[5] first described ice nucleating factors (INF's) from the hemolymph of freeze-tolerant beetles. These INF's «seed» hemolymph ice crystal formation at high subzero temperatures (circa −6 °C).INF's from two insect species have been purified and partially characterized as being proteins or lipoproteins[6,7]. Nucleating factors override the colligative properties of other compounds such as sugars, polyols, or salts which would normally depress hemolymph supercooling points. It has been proposed that seeding extracellular ice at a high subzero temperature promotes water movement from cells as water freezes in the extracellular spaces. The resulting cellular dehydration is thought to reduce the possibility of intracellular ice crystal formation, which would cause extensive cell damage. To date, INF's have been reported to occur in only three insect orders: Diptera, Coleoptera, and Hymenoptera[8,9]. However, on the basis of hemolymph supercooling point determinations (above −10 °C) the freeze-tolerant cockroach *Parcoblatta pennsylvanica*[10] appears to have hemolymph ice nucleators. This report suggests their occurrence in *P. pennsylvanica*, but no confirmation of their presence was provided.

Although INF's normally occur in hemolymph during the winter, there are reports that they may be found in insects during the summer. There is no clear explanation for the occurrence of INF's in summer-acclimated insects, but they may be present under circumstances where summer night temperatures drop below freezing[11,12], or the 'INF's' may have multiple functions as suggested by Duman et al.[7].

Cryptocercus punctulatus collected in November 1983 were found to be freeze-tolerant. Both whole insects and hemolymph plasma samples supercooled to similar points, ranging between −5.5 and −5.6 °C (table 1). The supercooling point was observed as a sudden rise in temperature (as measured by a 40-gauge copper-constantan thermocouple and a Leeds and Northrup Speedomax 250 recorder) due to the release of the latent heat of fusion of the solution as the temperature was slowly lowered (−0.3 °C/min). Cold-acclimated *C. punctulatus* frozen for relatively short time intervals invariably recover upon warming to room temperature. We have found 76% survival (N = 94) among individuals held up to 205 days at −10 °C when observed 3 days post-thaw.

Experientia 41 (1985), Birkhäuser Verlag, CH-4010 Basel/Switzerland

Ice nucleating acitivity was retained when summer- and winter-collected hemolymph serum was diluted 1:100 with distilled water (table 1). It can be seen from this table that most of the INF activity in plasma was lost upon heating at 100 °C for 2 min. In addition, mixing equal parts of *C. punctulatus* hemolymph and 1 M glycerol (0.5 M total) produced very little effect on the supercooling point of the samples. However, if hemolymph from lab-reared, noncold-acclimated *Periplaneta americana* is mixed in the same proportions, a shift is seen in the supercooling point (from −12.1 to −14.4 °C). These findings are in very close agreement with Zachariassens[5,9] working criterion for establishing nucleating activity in insect hemolymph.

Table 1. Supercooling point comparisons of whole body and 2-µl hemolymph plasma samples of *C. punctulatus*

Samples	Supercooling point (°C + SD)[a]
Winter-collected[b]	
Whole insect (N = 5)	−5.6 ± 0.3
Hemolymph (50 individuals)	−5.5 ± 0.0
1:10 dilution	−5.2 ± 0.7
1:100 dilution	−6.2 ± 0.6
Heat-treated hemolymph	−8.4 ± 2.1
Summer-collected[c]	
Whole insect (N = 5)	−5.8 ± 0.4
Hemolymph (50 individuals)	−5.2 ± 0.8
1:10 dilution	−5.5 ± 0.4
1:100 dilution	−6.6 ± 0.4
Heat-treated hemolymph	−9.5 ± 0.7
Distilled water	−12.8 ± 0.5
Periplaneta americana	−12.1 ± 2.7
P. americana + 0.5 M glycerol	−14.4 ± 3.8
C. punctulatus[b] + 0.5 M glycerol	−5.8 ± 0.6
1 M glycerol	Unfrozen at −17

[a] N = 5. [b] Collected November 29, 1984. [c] Collected July 24, 1984.

Table 2. R_f values of polyols on silica gel high performance thin layer chromatography plates

Compound/source	Solvent number[a]		
	1	2	3
Glycerol	0.58	0.65	0.50
C. punctulatus plasma	0.35	0.50	0.32
Ribitol	0.35	0.50	0.32
Arabitol	0.31	0.44	0.30
Xylitol	0.27	0.41	0.25

[a] Solvent 1: ethyl acetate:methanol:butanol:water (16:4:4:2). Solvent 2: butanol:acetone:water (5:4:1). Solvent 3: butanol:water (9:1).
10 × 10 cm prepared silica gel 60 F254 HPTLC plates (Merck) 1 µl spotted volumes.
Journal of Chromatography Library volume 9 (1977) HPTLC, High Performance Thin-Layer Chromatography edited by A. Zlatkis and R.E. Kaiser.

Table 3. Seasonality of ribitol concentrations in pooled adult *C. punctulatus* hemolymph

Collection date	Concentration mg/ml	mM
Nov. 29, 1983	13.9	91.3
Jan. 29, 1984	14.9	97.9
March 8, 1984	2.3	15.1
July 24, 1984	ND	—
Sept. 29, 1984	ND	—
Oct. 1, 1984	ND	—
Oct. 17, 1984	1.0	6.6
Nov. 12, 1984	1.7	11.2
Dec. 13, 1984	0.4	2.6
Insects held in cold storage[a]	9.6	63.1

ND = not detectable. [a] Cold storage: *C. punctulatus* collected on Nov. 12, 1984, and maintained in the dark at 0 °C for 1 month.

Insects collected in July 1984 were also found to contain hemolymph INF's, but were not freeze-tolerant. Freezing occurred at −5.8 °C, but these insects did not recover when warmed to room temperature. Since hemolymph INF's are present throughout the year in this species, it appears that other factors may be involved in providing for their freeze tolerance. These insects were collected at Mt Lake, Va, where summer temperatures do not drop below freezing. The reason as to why INF's are present in the hemolymph during the summer is not clear. However, since these insects feed on materials which are low in nitrogen, it is possible that the presence of the INF's may be the result of nitrogen economy (i.e. low levels of hemolymph protein turnover during seasonal temperature changes).

Various polyols have been found to occur in the hemolymph of freeze-tolerant insects[13,14]. Since nucleating factors override the antifreeze effects of the polyols, it is possible that the polyols act as cryoprotectants helping to stabilize protein structure, maintain membrane integrity, and 'buffer' against extreme osmotic fluctuations[14–16]. Five polyols ranging from 3 to 6 carbons have been previously reported in insect hemolymph. They are: glycerol, sorbitol, mannitol, threitol, and erythritol. However, an unidentified 5-carbon polyol – possibly ribitol, arabitol, or xylitol has been reported from whole body extracts of cold-acclimated collembolans[17]. Also, ribitol has been reported in Australian scale insect excreta which was thought to be plant derived[18]. Analysis of winter-acclimated *C. punctulatus* hemolymph using high performance thin layer chromatography (HPTLC) and several solvent systems, detected the presence of ribitol. Identical R_f values were obtained for ribitol standards and the hemolymph polyol (table 2). The identification of ribitol was confirmed by using two gas-liquid chromatography systems. The formation of polyol trimethylsilyl esters or acetate derivatives was performed as outlined by Laker[19]. Trimethylsilyl derivatives and a SE-30 column (oven temperature = 160 °C, isothermal) were used to determine that the hemolymph polyol was either ribitol or arabitol. Good separation/quantitation between ribitol and its two isomers xylitol and arabitol was achieved using acetate derivatives and an OV-225 column (oven temperature = 210 °C, isothermal). This is a new report of hemolymph-borne ribitol in insects. Its presence is seasonal (table 3) and may be associated with freeze-tolerance. This may explain why summer (July) insects are not freeze-tolerant. Since they contained INF's, and hemolymph ribitol is not detectable, both materials may be needed to provide for freeze-tolerance.

It is known that hemolymph polyol levels can fluctuate in response to variations in ambient temperatures[20]. Upon exposure to 'warm' temperatures, polyols can be lost in a matter of hours[13] or days[15,20]. Ribitol concentrations in field-collected insects decreased between 12 November 1984 (1.7 mg/ml) and 13 December 1984 (0.4 mg/ml) (table 3). This drop in ribitol levels was apparently due to an unusually warm period which occurred within the month prior to the December collection (13 December 1984). During that same interval, insects maintained at 0 °C in the dark showed a considerable increase in hemolymph ribitol concentration (63.1 mM). It appears that *C. punctulatus* might prove to be ideal organisms for detailed studies on freeze-tolerance. This is because of their relative large size (700 mg), abundance, longevity, and because of their dependence on gut symbiotes. It is possible that the symbiotic relationship may provide some assistance (i.e. production of ribitol/ribitol precursors) in the freeze-tolerance mechanism.

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1 Cleveland, L.R., Hall, S.R., Sanders, E.P., and Collier, J., Mem. Am. Acad. Arts Sci. *17* (1934) 185.
2 Martin, M., Comp. Biochem. Physiol. *75A* (1983) 313.
3 Nalepa, C.A., Behav. Ecol. Sociobiol. *14* (1984) 273.

4 Chino, H., Nature *180* (1957) 606.
5 Zachariassen, K.E., and Hammel, H.T., Nature *262* (1976) 285.
6 Duman, J.G., Morris, J.P., and Castellino, F.J., J. comp. Physiol. *154B* (1984) 79.
7 Duman, J.G., Neven, L.G., Beals, J.M., Olson, K.R., and Castellino, F.J., J. Insect Physiol. *31* (1985) 1.
8 Lee, R.E., Zachariassen, K.E., and Baust, J.G., Cryobiology *18* (1981) 511.
9 Zachariassen, K.E., Baust, J.G., and Lee, R.E., Cryobiology *19* (1982) 180.
10 Duman, J.G., J. Insect Physiol. *25* (1979) 805.
11 Zachariassen, K.E., Comp. Biochem. Physiol. *73A* (1982) 557.
12 Van der Laak, S., Comp. Biochem. Physiol. *73A* (1982) 613.
13 Baust, J.G., and Morrissey, R.E., Proc. XVth int. Congr. Ent. (1976) 173.
14 Baust, J.G., Comp. Biochem. Physiol. *73A* (1982) 563.
15 Zachariassen, K.E., Norw. J. Ent. *24* (1977) 25.
16 Zachariassen, K.E., J. Insect Physiol. *25* (1979) 29.
17 Block, W., and Zettel, J., Ecol. Ent. *5* (1980) 1.
18 Hackman, R.H., and Trikojus, V.M., Biochem. J. *51* (1952) 653.
19 Laker, M.F., J. Chromat. *184* (1980) 457.
20 Ring, R.A., and Tesar, D., J. Insect Physiol. *26* (1980) 763.

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Bronchial asymmetry and Fibonacci scaling

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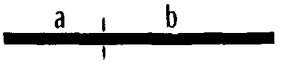
Summary. The irregular branching pattern of the bronchial tree in multiple mammalian species is consistent with a process of morphogenetic self-similarity described by Fibonacci scaling.
Key words. Bronchial tree; Fibonacci scaling; fractal; lung.

The bronchial system is a branching network of tubes. A key feature of this dendritic structure is its irregularity[1,2]. Over multiple generations, each tube gives rise to a conjugate pair of daughter structures of unequal lengths, beginning with the trachea which bifurcates into a long (left mainstem) and short (right mainstem) bronchus. Based on Weibel's data[2] (including figures 85 and 90) the ratio of the shorter to longer conjugate element, for the fifth through the seventh generations of the bronchial tree in a single human lung cast, was 0.62 ± 0.02 (mean ± SEM). The ratio of the right (~ 37 mm) to the left (~ 60 mm) mainstem bronchus was also ~ 0.62 in this cast.

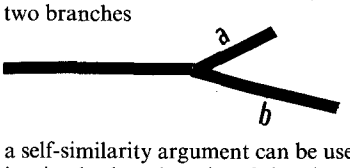
While the irregular pattern of branching of the human bronchial tree is well-recognized[1,2], its theoretical basis remains unexplained. We conjecture that this asymmetry may be a manifestation of the self-similarity principle of morphogenesis advanced by D'Arcy Thompson[3]. A simple model of one type of self-similar scaling can be obtained by starting with a rod of unit length and dividing it into two sections of respective lengths a and b such that the ratio of the total length (a+b) to the longer section b will be equal to the ratio b/a:



It is then readily demonstrated[4] that $(a+b)/b = b/a = (1+\sqrt{5})/2 \approx 1.62$ which is referred to as the golden mean or divine proportion and is designated as φ. Inversely, $a/b = 1/\varphi \approx 0.62$.

The same self-similarity ratio is obtained by generating a series of positive integers such that each integer is the sum of the two preceding integers: 1, 1, 2, 3, 5, 8, 13, 21, 34, ... These numbers are referred to as the Fibonacci numbers: the ratio of one number to its immediate predecessor approaches φ as a limiting value.

In the case of the bronchial tree, since each tube bifurcates into two branches



a self-similarity argument can be used to predict Fibonacci scaling in the length ratio of daughter tubes a and b. The ratios computed from Weibel's data, cited above, are suggestive in this regard.

To further test this hypothesis, data from Raabe et al.[5] which provide detailed morphometric measurements of silicone rubber bronchial casts from four mammalian species were analyzed. Computations were made from bronchial tree measurements of two adult male humans, two male beagle dogs, a female Long-Evans rat and a female Syrian hamster (table). We assessed the short:long branch length ratios in each cast by two methods. Method A: mean ±SEM values were computed from the ratio of short:long conjugate tube lengths for each bifurcation for which measurements were available in bronchial generations one (right and left mainstem bronchi) through seven of the bronchial tree. Method B: the sum of all short (Σ short) and all long (Σ long) branches in bronchial generations one through seven was obtained. The ratio Σ short/Σ long was then calculated for each generation and the overall mean ± SEM computed for these seven generations.

The data in the table along with Weibel's data are compatible with the hypothesis that the Fibonacci ratio is a universal scaling ratio in the bronchial tree. It should be emphasized that these values represent the mean ratios derived from multiple conjugate branch pairs in each of the human and animal lung casts. As expected, there is considerable biologic variability around the 'ideal' (i.e. average) ratio of 0.62 for any single branch pair ratio. The consistency of the mean ratio derived from four species, however, is highly suggestive of a Fibonacci scaling mechanism.

In a different analysis of bronchial structure, Horsfield[6,7] generated a Fibonacci sequence by using a numbering system for successive branches in a bifurcating tree. However, this model

Bronchial length ratios (computed from Raabe et al.[5])

Lung cast	Method A*	Method B* (n = 7)
Human HM-2-72	0.66 ± 0.02 (n = 109)	0.62 ± 0.03
Human HM-3-73	0.64 ± 0.02 (n = 117)	0.62 ± 0.03
Dog DN-2-72	0.67 ± 0.02 (n = 110)	0.68 ± 0.02
Dog DN-5-75	0.67 ± 0.02 (n = 104)	0.66 ± 0.03
Rat RF-3-72	0.63 ± 0.03 (n = 100)	0.55 ± 0.05
Hamster HaF-4-73	0.62 ± 0.02 (n = 107)	0.59 ± 0.07

*See text for details. For method A, n = total number of conjugate bronchial branch pairs for which data were available in the first seven generations of bronchial tubes. For method B, n = 7 generations. Values are given as mean ± SEM.